Chapter-V

Synthesis of some novel 1,2,3-triazolo tagged thieno [2,3-d]pyrimidine derivatives by Click chemistry.
Introduction:

One of the intensively developing areas in modern organic chemistry is the development of methods for the synthesis and functionalization of compounds having significant pharmacological properties and the prospect of subsequent use as active pharmaceutical substrates. Thus several heterocyclic compounds based on [1,2,3]-triazole are found to show wide range of activities. However, there is a growing demand for the synthesis of organic molecules in order to identify potential lead molecules which can selectively control the specific disease without side effects. Therefore, In this research program we have selected some of the promising ring systems based on [1,2,3]-triazole are important for pharmaceutical industry due to their applications and continue to appear since they exhibit a wide range of biological and medicinal activities. In this chapter, mainly aimed to developed for the synthesis of thienopyrimidine of morpholine/piperidine attach to [1,2,3]triazoles.

A large number of triazoles and thienopyrimidines have been prepared and many of these compounds have shown a wide spectrum of antimicrobial activity. Some triazoles/thienopyrimidines with different substituents at different location on the heterocyclic ring resulted in fungicidal and antibacterial activity of various potencies. Since their discovery during the 20th century, antimicrobial agents (antibiotics and related antimicrobial drugs) have substantially reduced the threat posed by infectious diseases. The use of these "wonder drugs", combined with improvements in sanitation, housing and nutrition and the advent of widespread immunization programme and the development of numbers of antimicrobial agents for treatment of microbial infections has led to a dramatic drop in deaths from diseases that were previously widespread, untreatable and frequently serious. The firm appearances of microbial infections followed by the spreading out of numerous resistant bacterial and fungal strains against clinically used antimicrobial have insist on medicinal communities to look for new incorporations into the current methods used in medicine. Severe probability of microbial infections along with immunosuppressive individuals due to the HIV infection, cancer treatments and organ transplantations actuated additional urgency to generate new antimicrobial agents. Moreover, in some cases especially in patients with impaired liver or kidney functions bring into play of antimicrobial drugs to pleasure infections causes several problems1-3. Thus, these trends have emphasized the urgent, innovative more helpful and safe antimicrobial agents. Along with the striking approaches to attain this
The development of structurally new classes of antimicrobial agents with novel mechanism of action and the other contained structural modification or optimization of the existing agents by improving both the binding affinity and spectrum of activity while retaining bioavailability and safety profile have provoked special interest in the area of medical chemistry. However, the increasing dominance of one such strategy that has been pursued in recent years employs a combination of two different active fragments in one molecule has emerged. With this strategy, each drug moiety is designed to bind independently two different biological targets and synchronously accumulate at mutually intention sites. Such twin exploit drugs or hybrid drugs suggest the possibility to overcome the current resistance and reduce the appearance of new resistant strains.

Richard Luke et al., reported the improvement in potency against Tie-2 of novel thienopyrimidine (1) and thiazolopyrimidine kinase inhibitors. The crystal structure of one of these compounds bound to the Tie-2 kinase domain is consistent with the SAR. These compounds have moderate potency in cellular assays of Tie-2 inhibition, good physical properties, DMPK and show evidence of in vivo inhibition of Tie-2.

Jeroen C et al., synthesized 2-aryl-4-morpholinothieno[3,2-d]pyrimidines (2) which are known as PI3K inhibitors. This class of compounds also potently inhibited the homologous enzyme mTOR. Replacement of the morpholine group in these compounds with 8-oxa-3-azabicyclo octane group led to mTOR inhibitors with selectivity over PI3K. Optimization of the 2-aryl substituent led to the discovery of 2-(4-ureidophenyl)-thienopyrimidines as highly potent (IC50<1nM) mTOR inhibitors with excellent selectivity (upto >1,000-fold) over PI3K and good potency in a cellular proliferation assay (IC50<50 nM).
Roger J Gillespie et al.,\textsuperscript{8} reported that the (-)-(11R, 2S)-enantiomer of the antimalarial drug mefloquine has been found to be a reasonably potent and moderately selective adenosine A\textsubscript{2A} receptor antagonist. Further investigation of this compound has led to the discovery of a series of keto-aryl thieno [3,2-\textit{d}]pyrimidine (3) derivatives, which are potent and selective antagonists of the adenosine A\textsubscript{2A} receptor. These derivatives show selectivity against the A\textsubscript{1} receptor. Furthermore, some of these compounds have been shown to have \textit{in vivo} activity in a commonly used model suggesting the potential for the treatment of Parkinson’s disease.

![Chemical Structure](Image)

\[ R = \text{CF}_3, \text{Me}, \text{OMe}, \text{NH}_2, \text{NH}_2-\text{NH}_2 \]

Alagarsamy et al.,\textsuperscript{9} designed and synthesized some of 2-methylthio-3-substituted-5,6-dimethylthieno[2,3-\textit{d}]Pyrimidin-4(3\textit{H})-ones (4). The synthesized compounds were investigated for analgesic, anti-inflammatory and antibacterial activities. While the test compounds exhibited significant activity the compounds showed more potent analgesic activity, and the compounds showed anti-inflammatory activity comparable to the reference standard diclofenac.

![Chemical Structure](Image)

\[ R^1 = \text{H}, \text{NHCH}_3, \text{N(CH}_3)_2 \]

Ogawva K et al.,\textsuperscript{10} synthesized a series of 2,4-dioxo-thieno[2,3-\textit{d}], [3,2-\textit{d}] and [3,4-\textit{d}] pyrimidin-1-acetic acids (5) with a benzyl moiety at the N-3 position and tested \textit{in vitro} for aldose reductase inhibitory activity against partially purified enzyme from rat lens. Some of these compounds were also evaluated for inhibition of sorbitol accumulation in the sciatic nerve or lens of streptozotocin-induced diabetic rats \textit{in vivo}. Among the synthesized compounds, several showed potent aldose reductase inhibitory activity with IC\textsubscript{50} in the 10\textsuperscript{-8}M range. Particularly the potencies of non-substituted
thieno-5-methylthieno, 5,6-dimethylthieno 6-isopropylthieno, 6-chloro thienopyrimidine and benzo thienopyrimidine analogs were approximately equipotent to FK-366 and Ponalrestat as references. Although most compounds were inactive in vivo compounds possessed moderate in vivo activity.

Wufu Zhu et al., synthesized the series of thieno-pyrimidine morpholine derivatives (6) bearing chromone moieties and evaluated for inhibitory activity against mTOR kinase, PI3Ka kinase and cytotoxicity of two cancer cell lines in vitro. The pharmacological results indicated that two compounds inhibited mTOR at lower doses and were more cytotoxic than lead compound 1. And three compounds also displayed moderate to excellent PI3Ka kinase inhibition activity with IC\textsubscript{50} values from 1.805 mM to 2.352 mM.

Manal M. Kandeel et al., reported anticancer activity of thieno[2,3-d]pyrimidines a series of 24 new 2-substitutedhexahydrocycloocta[4,5] thieno[2,3-d]pyrimidines with different substituents at C-4 position and hexahydrocycloocta[4,5] thieno[3,2-e]-1,2,4-triazolo[4,3-c]pyrimidines (7) were synthesized The thienotriazolopyrimidine derivatives showed broad spectrum potent anticancer activity in nano molar to micro molar range against 56 human tumor cell lines with GI\textsubscript{50} ranging from 0.495 to 5.57 mM.
Additionally, Click chemistry has become a unique and efficient modern chemistry technique for chemical synthesis of thienopyrimidine molecules. Click chemistry deals with beautifully represented among cycloaddition reactions involving 1,3-dipolar cycloaddition. Click reactions can be utilized to construct building blocks for the rapid synthesis of molecules with diverse structure and function. Synthesis of [1,2,3]triazoles is mainly the copper(I) catalyzed 1,2,3-triazole (8) forming reaction between azides and terminal alkynes is called ‘click chemistry’.

\[
R^1-N_3 + \equiv-R^2 \xrightarrow{\text{Cu}^1} R^1-N=N-R^2
\]

1,4-Disubstituted [1,2,3]-triazole

In this respect, the classic 1,3-dipolar cycloaddition fails as a true click reaction. A copper-catalyzed variant that follows a different mechanism can be conducted under aqueous conditions, even at room temperature. Additionally, the classic Huisgen 1,3-dipolar cycloaddition often gives mixtures of regioisomers, and the copper-catalyzed reaction allows the synthesis of the 1,4-disubstituted (9) regioisomers specifically. By contrast, a later developed ruthenium-catalyzed reaction gives the opposite regioselectivity with the formation of 1,5-disubstituted triazoles (10). Thus, these catalyzed reactions comply fully with the definition of click chemistry and have put a focus on azide-alkyne cycloaddition as a prototype click reaction.
The outstanding catalytic performance of Cu$_2$O under aqueous environment led to investigate the effect of water, as well as the possibility of the amount of catalyst used in the transformation. Good yields of triazole (11) can be detected from the reaction mixture of alkyne with tosylazide in the presence of 10 mol% Cu$_2$O under neat conditions in 6 h$^{13}$. 

The synthesis of [1,2,3]-triazole-4-yl- methanol involved the 1,3-dipolar cycloaddition reaction between propargyl alcohol and aromatic azides catalyzed by Cu(I) to obtain respective [1,2,3]-triazole derivatives (12)$^{14}$. This cycloaddition reactions were performed under magnetic stirring at room temperature and were protected from light due to the photosensitivity of the aromatic azides. After purification in a flash-type column, triazole compounds were obtained as white or yellow crystals with yields ranging from 50% to 82%.

The importance of triazole derivatives lies in the field that these have occupied a unique position in heterocyclic chemistry, due to its various biological activities. [1,2,3]-Triazoles constitute an important class of nitrogen heterocycles in the field of organic and medicinal chemistry. Medicinally, they have been shown to possess a wide range of diverse interesting pharmacological properties such as antituberculosis$^{15}$, anti-HIV$^{16}$, antimalarial$^{17}$, antiepileptic$^{18}$, antiallergic$^{19}$, antileishmanial$^{20}$, antifungal$^{21,22}$, anticancer$^{23,24}$ and
antibacterial activities. In addition, these molecules have been utilized as proton transport facilitators, glycoside cluster arrays, spacers or linkers to dendrimers, DNA cleaving agents, structural components in hyper branched polymers and most importantly in liquid crystals. There are very few [1,2,3]-triazole containing molecules on the market in the last stage of clinical trials. Potential pharmaceuticals based on [1,2,3]-triazoles include the anticancer compound carboxyamidotriazole (CAI) (13), the nucleoside derivative non-nucleoside reverse transcriptase inhibitor tert-butyldimethylsilyl spiroamino xanthioledioxide (known as TSAO) (14), β-lactam antibiotic tazobactum (15), cephalosporine cefatrizine (16) and so on.

The emerging field of click chemistry offers a unique approach to the synthesis of [1,2,3]-triazole containing molecules. This reaction owes its usefulness in part to the ease with which azides and alkynes can be introduced into a molecule and their relative stability under a variety of conditions. Azides and alkynes are essentially inert to most biological and organic conditions, molecular oxygen, water and the majority of common reaction conditions in organic synthesis. [1,2,3]-Triazole moieties are attractive connecting units because they are stable to metabolic degradation and capable of hydrogen bonding, which can be favourable in the binding of bio-molecular targets and can improve the solubility. The [1,2,3]-triazole moiety does not occur in nature, although the synthetic molecules that contain [1,2,3]-triazole units show diverse biological activities. The importance of triazole compounds in medicinal chemistry is
undeniable. Based on the importance, we have exemplified the 1,2,3-triazole molecules (17-22) according to their biological activities^{39-46}.

H. A. Aisa et al.,^{47} two series of rupestonic acid derivatives, (1-substituted-1H-1,2,3-triazol-4-yl)methyl 2-((5R,8S,8aS)-3,8-dimethyl-2-oxo-1,2,4,5,6,7,8,8a-octahydroazulen-5-yl)acrylate (25a) and N-(1-substituted-1H-1,2,3-triazol-4-yl)methyl 2-((5R,8S,8aS)-3,8-dimethyl-2-oxo-1,2,4,5,6,7,8,8a-octahydroazulen-5-yl)acrylamide (25b) were easily and efficiently synthesized via click chemistry. These compounds were tested for their in vitro activities against various strains of influenza A virus (H1N1, oseltamivir resistant H1N1, H3N2) and influenza B virus. The results showed that nine compounds were active against the H1N1 strain of influenza A virus.
Z. Y. Wang et al.,\textsuperscript{48} and co-workers have been synthesized a series of novel chiral 2(5H)-furanone derivatives containing 1,2,3-triazole moiety (26) with potential biological activity were designed and synthesized from available (5S)-5-alkoxy-3,4-dibromo-2(5H)-furanones, amino acids, propargyl bromide and organic azides through an asymmetric Michael addition-elimination, substitution and cycloaddition under mild conditions with economical catalysts. They also could be generated via a simple and efficient multi-component one-pot approach. Due to the diversity of 5-alkoxy-3,4-dihalo-2(5H)-furanones amino acids and organic azides.

B. A. Bhat et al.,\textsuperscript{49} have been designed and synthesized a series of ursolic acid-1-phenyl-1H-[1,2,3]triazol-4-ylmethylester (27) and to develop potent antitumor agents. A
regioselective approach using Huisgen 1,3-dipolar cycloaddition reaction of ursolic acid-alkyne derivative with various aromatic azides was employed to target an array of triazolyl derivatives in an efficient manner. All the compounds were evaluated for anticancer activity against a panel of four human cancer cell lines including A-549 (lung), MCF-7 (breast), HCT-116 (colon), THP-1 (leukemia) and a normal human epithelial cell line (FR-2) using sulforhodamine B assay. The pharmacological results showed that most of the compounds displayed high level of antitumor activities against the tested cancer cell lines compared with ursolic acid.

Apart from these [1,2,3]triazolo ring system is known to be associated with wide range of therapeutic activities such as anticancer, antitubercular, antihypertensive and VEGF receptor tyrosine kinase inhibitor activities. In spite of therapeutic significance of [1,2,3]triazolo ring system, there are hardly few reports on synthesis and biological and pharmacological evaluation of compounds which contain thienopyrimidine and [1,2,3]triazolo moiety. This inspired us to take up the synthesis of novel compounds which contain both thienopyrimidine attach morpholine and [1,2,3]triazolo ring system and were evaluate their biological and pharmacological profile.
Present work

Since our aim was to synthesize thieno[2,3-\(d\)]pyrimidine[1,2,3]triazole derivatives (7\(a\)-f/8\(a\)-f). A synthetic analysis of these molecules suggested that an amino, nitrile thiophen ester ring would be prepared first, using the standard Gewald reaction. It was reacted with formic acid then converted into ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-\(d\)]pyrimidine-6-carboxylate (2) followed by chlorination in the presence of few drops of phosphorus oxychloride gives chloro compound (3) was protected with secondary amines to give ethyl 5-methyl -4-(substituted pipiridine1-yl/marpholino)thieno[2,3-\(d\)]pyrimidine active pharmacophore groups, i.e. thieno[2,3-\(d\)]pyrimidine moiety and 1,2,3-trizole nucleus, we employed -6-carboxylate (4) was treated with aq. NaOH and MeOH medium to give ethyl 5-methyl -4-(substituted pipiridine/marpholin 1-yl)thieno[2,3-\(d\)]pyrimidine-6-carboxylic acid derivatives (5\(a\)-b). The acid compound was treated with propargyl bromide in the presence of K\(_2\)CO\(_3\), DMF to afforded compound (6). Further alkyne compounds on treatment with appropriate substituted arylazides in the presence of CuSO\(_4\).5H\(_2\)O/Na-Ascorbate in THF/H\(_2\)O (1:1) yielded the title compounds (7\(a\)-f/8\(a\)-f) in good to excellent yields. The synthesized compounds were screened for in vitro antimicrobial property by standard methods. The work carried out in this chapter is described in the following steps.

2. Conversion of compound 1 into esters of thieno[2,3-\(d\)]pyrimidine 2.
3. Chlorination of ester functions of compound 2 into corresponding ester of substituted 4- chloro thieno[2,3-\(d\)]pyrimidine 3.
4. Ester of 4-chlorothieno[2,3-\(d\)]pyrimidine 3 conversion into 4-morpholino/4-(piperidin-1-yl)thieno[2,3-\(d\)]pyrimidine 4.
5. Conversion of ester functions of compound 4 into corresponding carboxylic acid derivatives 5.
6. Propargylation of the acid compound 5 to afforded thieno[2,3-\(d\)]pyrimidine alkyne compounds 6.
7. Synthesis of target compounds 7/8 from alkyne 6 with various substituted arylazides by Click-chemistry

Series of reactions which are carried out to get the target molecules are depicted in scheme V.
### Scheme V

**Reagents and conditions:**
- a) HCOOH, Reflux, Over night;
- b) POCl₃, Reflux 3 h;
- c) Morpholine/piperidine, ethanol, Et₃N, 2 h;
- d) NaOH, MeOH, rt, 2 h;
- e) propargyl bromide; K₂CO₃, DMF, 2 h, rt;
- iii) sub-Ar-N₃, CuSO₄.5H₂O/Na-Ascorbate, THF/H₂O (1:1), 10 min, rt.

1. **Synthesis of ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate 1**

   Compound ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate (1) was synthesized via single step process of Gewald method. The solution containing ethyl acetoacetate, malononitrile, elemental sulfur in DMF and catalytic amount of triethylamine was heated to get compound 1.

2. **Conversion of compound 1 into esters of thieno[2,3-\text{d}] pyrimidine 2**

   Ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate (1) was refluxed with formic acid to afforded corresponding ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-\text{d}]pyrimidine-6-carboxylate 2.
The IR spectra (Fig.5.1.) of the compound 2 exhibited peaks at 3386, 1685 and 1580 cm$^{-1}$ corresponding to the stretching frequencies of NH, C=O and C=N groups respectively. Its $^1$H-NMR spectra (Fig.5.1.1.) exhibited singlets at 5.06 and 8.25 ppm due to one proton of NH and pyrimidine CH groups, quartet at 4.25 ppm due to two protons, triplet at 1.25 ppm of three protons of ester group of thieno[2,3-d]pyrimidine nucleus.

3. Chlorination of ester functions of compound 2 into corresponding ester of substituted 4-chlorothieno[2,3-d] pyrimidine 3

Compound 2 was treated with phosphorous oxychloride to afford the corresponding chloro derivative ethyl 4-chloro-5-methylthieno[2,3-d]pyrimidine-6-carboxylate 3.

The IR spectra of the compound 3 exhibited peaks at 1710 and 1541 cm$^{-1}$ corresponding to exhibited singlets at 5.06 at NH proton was disappered and 8.39 ppm and pyrimidine CH proton of 4-Chlorothieno[2,3-d]pyrimidine.

4. Ester of 4-Chlorothieno[2,3-d] pyrimidine compound conversion into 4-morpholino/4- (piperidin-1-yl)thieno[2,3-d] pyrimidines 4a-b

The compound 3 was refluxed with selected secondary amines (morpholine/piperidine) in absolute ethanol and catalytic amount of triethylamine to obtained compounds 4a-b.
The IR spectra of 4a showed the presence of C-N bands at 1531 cm\(^{-1}\). The \(^1\)H NMR spectra (Fig.5.2.1.) of 4a showed signals for the protons of the substituted secondary amino function at the 4-position in addition to the signals for multiplet at 1.63 ppm of six protons and triplet at 3.59 ppm of four protons of 4-substituted pyrroline.

**5. Conversion of ester functions of compound 4a-b into corresponding carboxylic acid derivatives 5a-b**

Compounds 4a-b on base hydrolysis in presence of NaOH in methanol as solvent at room temperature furnished their corresponding carboxylic acid derivatives 5a-b.

The IR spectrum (Fig.5.3.) of 5b exhibited characteristic peak at 3488, 1680 cm\(^{-1}\) for carbonyl of carboxylic acid stretching. \(^1\)H NMR spectrum of 5b (Fig.5.3.1.) showed a broad singlet at \(\delta\) 12.6 ppm corresponding to –COOH and two triplets at \(\delta\) 3.69 ppm and \(\delta\) 3.80 ppm of 4-substituted morpholine N–attached methylene of four protons and O–attached of four protons of corresponding compound 5b.

**6. Propargylation of the acid compound 5a-b to afforded thieno[2,3-d]pyrimidine alkyne compounds 6a-b**

Formation of alkyne terminated compound 5a-b was carried out by reacting 5-methyl-4-morpholino/piperdineothieno[2,3-d]pyrimidine-6-carboxylic acid with propargyl bromide and \(\text{K}_2\text{CO}_3\) in DMF to yielded 5-methyl-4-morpholinothieno[2,3-d]pyrimidine propargyl ester 6a-b.
The $^1$H NMR spectra of compound 6a was showed two singlets at 3.645 and 4.97 ppm due to the $\equiv$CH and CH$_2$ protons of propargyl bromide (Fig.5.4.1.). Hence these signals confirmed the condensation of propargyl bromide.

7. Synthesis of target compounds 7a-f/8a-f from alkyne 6 with various substituted arylazides by Click-chemistry

Click Reaction: The azides (i-vi) and alkyne 6a-b cycloaddition reaction was successfully carried out to form 1,2,3- triazole ring through 1,3-diopolar cycloaddition mechanism, the synthesis of 1,4-disubstituted-1,2,3-triazoles was carried out using the copper (II) catalyzed cycloaddition reaction as follows The formation of the triazole was observed 7a-f and 8a-f in good yields.

The Click product 7a-f/8a-f was fully characterised by $^1$H NMR. The formation of 1,2,3-triazole ring was confirmed by the appearance of a clear characteristic peak in the $^1$H-NMR spectrum at 8.54 ppm for the triazole’s proton of compound 7d (Fig.5.6.1.).

EXPERIMENTAL

Synthesis of ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate 1

The starting ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate (1) was prepared according to Gewald synthetic procedure.

General procedure for the synthesis of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate 2

Compound 1 (2.59 g, 0.01 mol) in formic acid (5 mL) was refluxed for 12 h (TLC check, chloroform:methanol, 8:1). On cooling to room temperature the obtained solid product 2 was collected by vacuum filtration, washed thoroughly with water, dried and purified by column chromatography eluting with chloroform:methanol (8:1) to give pale brown crystals. Obtained yield 2.44 g (80%).
Yield (80%); IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3386, 2925, 1685, 1580; $^1$H NMR (DMSO $d_6$, 400 MHz) $\delta$ 1.25 (t, 3H, CH$_2$-$\text{CH}_3$), 2.80 (s, 3H, thiophene-$\text{CH}_3$), 4.25 (q, 2H, -CH$_2$-$\text{CH}_3$), 5.06 (s, 1H, NH). 8.25 (s, 1H, pyrimidine-CH); $^{13}$C NMR (CDCl$_3$,100 MHz) $\delta$ 14.04, 14.74, 61.02, 121.41, 123.70, 143.24, 148.16, 158.17, 161.72, 165.67; LC–MS (positive ion mode): m/z 239 (M+H)$^+$ for C$_{10}$H$_{10}$N$_2$O$_3$S.

**General procedure for the synthesis of ethyl 4-chloro-5-methylthieno[2,3-d]pyrimidine-6-carboxylate 3**

A mixture of compound 3 (0.01 mol) and phosphorus oxychloride (20 mL) was refluxed for 12 h. The excess of phosphorus oxychloride was distilled off under reduced pressure and the residue thus obtained was treated with sodium bicarbonate solution (10%). The resulting solid was collected, washed with water, dried and recrystallized from ethanol to give compound 3.

Yield (81%); IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 2918, 1710, 1541; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.68 (t, 3H, CH$_2$-$\text{CH}_3$), 2.73 (s, 3H, thiophene-$\text{CH}_3$), 4.12 (q, 2H, -CH$_2$-$\text{CH}_3$), 8.39 (s, 1H, pyrimidine-CH); $^{13}$C NMR (CDCl$_3$,100 MHz) $\delta$ 14.14, 14.85, 61.09, 75.68, 121.51, 123.90, 143.54, 148.19, 158.08, 159.72, 166.92; LC–MS (positive ion mode): m/z 257 (M+H)$^+$ for C$_{10}$H$_9$ClN$_2$O$_2$S.

**General procedure for the synthesis of compounds 4a-b**

A mixture of 3 (0.001 mol), the selected secondary amine (0.001 mol) and triethylamine (0.36 mL, 0.003 mol) in absolute ethanol (12 mL) was stirred for 2-3 h, after cooling the separated solid was filtered, dried and crystallized from ethanol.

**Ethyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 4a**

Yield (72%); IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 2980, 2851, 1703, 1531; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.34 (t, 3H, CH$_2$-$\text{CH}_3$), 1.63 (m, 6H, piperdine-$\text{CH}_2$), 2.73 (s, 3H, thiophene-$\text{CH}_3$), 3.59 (t, 4H, piperdine-$\text{CH}_2$), 4.35 (q, 2H, -CH$_2$-$\text{CH}_3$), 8.60 (s, 1H, pyrimidine-CH); $^{13}$C NMR (DMSO–$d_6$,100 MHz) $\delta$ 14.08, 16.39, 23.58, 25.10, 50.80, 61.15, 119.20, 121.93, 139.66, 153.76, 162.02, 162.85, 168.76; LC–MS (positive ion mode): m/z 306 (M+H)$^+$ for C$_{15}$H$_{19}$N$_3$O$_2$S. C
General procedure for the synthesis of 5 compounds 5a-b

The compound 4a-b was dissolved in MeOH/ H2O (8 mL: 4 mL), and 15% v/v NaOH aq (2 mL) was added. Stirring was continued for 6 h at rt, then CHCl3 was added. The aqueous layer was acidified with 1 N HCl, stirred for 15 min, the product was separated by vacuum filtration and washed with water, dried well and recrystallized from chloroform and methanol to give compounds 5a-b in good yield.

5-Methyl-4-morpholinothieno[2,3-d]pyrimidine-6-carboxylic acid 5b

Yield (80%); IR (KBr) (vmax/cm⁻¹): 3488, 2984, 1680, 1541; ¹H NMR (CDCl3, 400 MHz) δ 2.78 (s, 3H, thiophene-CH3), 3.69 (d, 4H, morphiline-CH2), 3.80 (d, 4H, morphiline-CH2), 8.60 (s, 1H, pyrimidine-CH), 12.6 (brs, 1H, -COOH). ¹³C NMR (CDCl3, 100 MHz) δ 16.14, 50.39, 65.71, 119.83, 124.46, 138.27, 153.63, 162.79, 163.63, 167.86; LC–MS (positive ion mode): m/z 280 (M+H)+ for C12H13N3O3S.

General procedure for the synthesis of 6a-b

Compounds 5a-b (3 mmol) and potassium carbonate (6 mmol) in 1:2 ratio were taken in 20 ml of DMF in two necked flask. To this mixture propargyl bromide was added drop wise under heating and refluxed for 3 hours and the mixture was concentrated in vacuum and added to the ice cold water. The obtained propargylated product was collected by filtration and separated by column chromatography.

Prop-2-yn-1-yl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 6a

Yield (70%); IR (KBr) (vmax/cm⁻¹): 2849, 1707, 1540; ¹H NMR (CDCl3, 400 MHz) δ 1.63 (m, 6H, piperidine-CH2), 2.73 (s, 3H, thiophene-CH3), 3.46 (t, 4H, piperidine-CH2), 3.64 (s, 1H, ≡CH), 4.97 (s, 2H, C≡CH2), 8.57 (s, 1H, pyrimidine-CH); ¹³C NMR (CDCl3, 100 MHz) δ 16.76, 24.15, 25.54, 51.49, 52.47, 75.28, 77.33, 120.00, 121.80, 141.00, 154.15, 161.98, 163.77, 169.19; LC–MS (positive ion mode): m/z 316 (M+H)+ for C16H17N3O2S.

Prop-2-yn-1-yl 5-methyl-4-morpholinothieno[2,3-d]pyrimidine-6-carboxylate 6b

Yield (82%); IR (KBr) (vmax/cm⁻¹): 2890, 1709, 1564; ¹H NMR (CDCl3, 400 MHz) δ 2.55 (t, 4H, morpholine-CH2), 2.84 (s, 3H, thiophene-CH3), 3.55 (t, 4H, morpholine-CH2), 3.86 (s, 1H, ≡CH), 4.93 (s, 2H, C≡CH2), 8.62 (s, 1H, pyrimidine-CH); ¹³C NMR (CDCl3, 100 MHz) δ 16.64, 25.15, 50.77, 52.59, 66.35,
75.40, 120.13, 122.89, 140.01, 154.12, 161.78, 163.44, 169.30; LC–MS (positive ion mode): m/z 318 (M+H)+ for C_{13}H_{15}N_{3}O_{3}S.

**General procedure for the synthesis of (7a-f) and (8a-f)**

The compounds 6a-b (1 mmol) and various aromatic azide (2 mmol) were suspended in THF (10 ml). Sodium ascorbate (0.3 mmol, in water) was added, followed by copper (II) sulphate pentahydrate (0.03 mmol, in water). The heterogeneous mixture was stirred vigorously 10 min, and the completion of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with cooled ice water. The crude triazolyl thienopyrimidine product was collected by extraction with ethyl acetate. The product was purified by column chromatography.

**(1-p-Tolyl-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 7a**

Yield (71%); IR (KBr) (ν\textsubscript{max}/cm\textsuperscript{-1}): 2950, 1712, 1542; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) δ 1.68 (m, 6H, piperidine-CH\textsubscript{2}), 2.31 (s, 3H, CH\textsubscript{3}), 2.62 (s, 3H, thiophene-CH\textsubscript{3}), 3.68 (t, 4H, piperidine-CH\textsubscript{2}), 5.56 (s, 2H, CH\textsubscript{2}), 7.62 (d, 1H, Ar-H), 7.93 (d, 1H, Ar-H), 8.36 (s, 1H, triazole-CH), 8.98 (s, 1H, pyrimidine-H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 100 MHz); δ 16.25, 21.22, 29.35, 39.22, 46.41, 107.26, 112.26, 119.18, 123.42, 126.62, 129.18, 132.44, 136.20, 139.43, 143.02, 148.21, 149.84, 156.51, 165.21; LC–MS (positive ion mode): m/z 449 (M+H)+ for C_{23}H_{24}N_{6}O_{2}S.

**(1-(4-Methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 7b**

Yield (69%); IR (KBr) (ν\textsubscript{max}/cm\textsuperscript{-1}): 2923, 1708, 1531; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) δ 1.86 (m, 6H, piperidine-CH\textsubscript{2}), 2.62 (s, 3H, thiophene-CH\textsubscript{3}), 3.91 (s, 3H, OCH\textsubscript{3}), 4.11 (s, 3H, CH\textsubscript{3}), 5.56 (s, 2H, CH\textsubscript{2}), 7.86 (d, 1H, Ar-H), 8.12 (d, 1H, Ar-H), 8.86 (s, 1H, triazole-CH), 9.14 (s, 1H, pyrimidine-H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 100 MHz); δ 17.35, 26.44, 29.32, 40.16, 46.24, 112.57, 119.31, 120.27, 125.18, 128.06, 130.76, 133.67, 138.26, 140.27, 146.17, 148.27, 149.16, 159.57, 167.19; LC–MS (positive ion mode): m/z 465 (M+H)+ for C_{23}H_{24}N_{6}O_{3}S.
(1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d] pyrimidine-6-carboxylate 7c

Yield (88%); IR (KBr) \( (\nu_{\text{max}}/\text{cm}^{-1}) \): 2822, 1704, 1545; \(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.59 (m, 6H, piperdine-CH\(_2\)), 2.80 (s, 3H, thiophene-CH\(_3\)), 3.52 (t, 4H, piperdine-CH\(_2\)), 5.53 (s, 2H, CH\(_2\)), 7.52 (d, 1H, Ar-H), 7.70 (d, 1H, Ar-H), 8.17 (s, 1H, triazole-CH), 8.58 (s, 1H, pyrimidine-H); \(^{13}\)C NMR (CDCl\(_3\),100 MHz); \( \delta \): 14.36, 28.42, 39.22, 68.41, 101.26, 117.26, 121.18, 135.42, 138.62, 141.18, 145.44, 148.20, 151.43, 156.02, 161.21, 166.84, 167.51, 169.21; LC–MS (positive ion mode): m/z 469 (M+H)+ for C\(_{22}\)H\(_{21}\)ClN\(_6\)O\(_2\)S.

(1-(3-Nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d] pyrimidine-6-carboxylate 7d

Yield (79%); IR (KBr) \( (\nu_{\text{max}}/\text{cm}^{-1}) \): 2978, 2850, 1705, 1531; \(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 1.71 (m, 6H, piperdine-CH\(_2\)), 2.81 (s, 3H, thiophene-CH\(_3\)), 3.49 (t, 4H, piperdine-CH\(_2\)), 5.57 (s, 2H, CH\(_2\)), 7.76 (t, 1H, Ar-H), 8.21 (d, 1H, Ar-H), 8.27 (s, 1H, Ar-H), 8.33 (d, 1H, Ar-H), 8.54 (s, 1H, triazole-CH), 8.62 (s, 1H, pyrimidine-H); \(^{13}\)C NMR (CDCl\(_3\),100 MHz; \( \delta \): 15.82, 23.20, 24.55, 28.66, 50.46, 119.83, 121.18, 124.83, 125.91, 127.81, 130.80, 131.45, 132.64, 133.89, 139.74, 141.89, 153.12, 161.62, 162.75, 168.12; LC–MS (positive ion mode): m/z 480 (M+H)+ for C\(_{22}\)H\(_{21}\)N\(_7\)O\(_4\)S.

(1-(3-(Trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno [2,3-d]pyrimidine-6-carboxylate 7e

Yield (69%); IR (KBr) \( (\nu_{\text{max}}/\text{cm}^{-1}) \): 2925, 1704, 1565; \(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \): 2.12 (s, 3H, thiophene-CH\(_3\)), 2.56 (m, 6H, piperdine-CH\(_2\)), 3.82 (t, 4H, piperdine-CH\(_2\)), 5.54 (s, 2H, CH\(_2\)), 7.14 (t, 1H, Ar-H), 7.26 (d, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 8.15 (d, 1H, Ar-H), 8.35 (s, 1H, triazole-CH), 8.92 (s, 1H, pyrimidine-H); \(^{13}\)C NMR (CDCl\(_3\),100 MHz; \( \delta \): 15.12, 28.22, 46.12, 54.14, 69.37, 117.85, 119.37, 121.40, 125.65, 127.13, 129.26, 130.24, 133.76, 133.89, 138.42, 140.23, 147.20, 151.03, 154.72, 162.42, 167.34; LC–MS (positive ion mode): m/z 503 (M+H)+ for C\(_{23}\)H\(_{21}\)F\(_3\)N\(_6\)O\(_2\)S.
(1-(3-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d] pyrimidine-6-carboxylate 7f

Yield (73%); IR (KBr) \((\nu_{\text{max}}/\text{cm}^{-1})\): 2856, 1714, 1521; \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 2.65 (s, 3H, thiophene–CH\(_3\)), 2.71 (m, 6H, piperdine–CH\(_2\)), 3.96 (t, 4H, piperdine–CH\(_2\)), 5.56 (s, 2H, CH\(_2\)), 7.06 (t, 1H, Ar-H), 7.48 (d, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 8.01 (d, 1H, Ar-H), 8.23 (s, 1H, triazole–CH), 8.74 (s, 1H, pyrimidine–H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz; \(\delta\) 16.64, 49.22, 58.12, 66.14, 111.37, 119.85, 120.37, 124.65, 126.00, 129.55, 131.04, 133.35, 133.74, 139.86, 140.17, 148.92, 151.03, 154.72, 162.42, 167.34; LC–MS (positive ion mode): m/z 453 (M+H\(^+\)) for C\(_{22}\)H\(_{21}\)FN\(_6\)O\(_2\)S.

(1-p-Tolyl-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d] pyrimidine-6-carboxylate 8a

Yield (72%); IR (KBr) \((\nu_{\text{max}}/\text{cm}^{-1})\): 2890, 1725, 1514; \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 2.16 (s, 3H, thiophene–CH\(_3\)), 2.88 (s, 3H, CH\(_3\)), 3.45 (t, 4H, morpholine–CH\(_2\)), 4.12 (t, 4H, morpholine–CH\(_2\)), 5.58 (s, 2H, CH\(_2\)), 7.48 (d, 2H, Ar-H), 7.84 (d, 2H, Ar-H), 8.36 (s, 1H, triazole–CH), 9.05 (s, 1H, pyrimidine–H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz; \(\delta\) 17.26, 23.52, 26.16, 39.22, 50.18, 65.24, 116.24, 119.31, 120.78, 125.64, 129.04, 132.47, 134.47, 139.16, 141.52, 152.01, 159.25, 165.24; LC–MS (positive ion mode): m/z 451 (M+H\(^+\)) for C\(_{22}\)H\(_{22}\)N\(_6\)O\(_3\)S.

(1-(4-Methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d] pyrimidine-6-carboxylate 8b

Yield (76%); IR (KBr) \((\nu_{\text{max}}/\text{cm}^{-1})\): 2869, 1650, 1520; \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 2.45 (s, 3H, thiophene–CH\(_3\)), 3.82 (t, 4H, morpholine–CH\(_2\)), 3.96 (t, 3H, OCH\(_3\)), 4.06 (t, 4H, morpholine–CH\(_2\)), 5.56 (s, 2H, CH\(_2\)), 7.62 (d, 2H, Ar-H), 7.99 (d, 2H, Ar-H), 8.30 (s, 1H, triazole–CH), 8.92 (s, 1H, pyrimidine–H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz; \(\delta\) 14.88, 21.50, 26.30, 48.60, 51.22, 68.02, 114.14, 119.35, 121.43, 124.44, 127.99, 130.46, 134.62, 138.16, 143.52, 154.01, 167.25, 169.16; LC–MS (positive ion mode): m/z 467 (M+H\(^+\)) for C\(_{22}\)H\(_{22}\)N\(_6\)O\(_4\)S.
Chapter-V

(1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8c

Yield (71%); IR (KBr) (ν_{max}/cm^{-1}): 2854, 1703, 1532; \(^1\)H NMR (CDCl\(_3\), 400 MHz) δ 2.68 (s, 3H, thiophene−CH\(_3\)), 3.84 (t, 4H, morpholine-CH\(_2\)), 4.13 (t, 4H, morpholine-CH\(_2\)), 5.50 (s, 2H, CH\(_2\)), 7.51 (d, 2H, Ar-H), 7.73 (d, 2H, Ar-H), 8.12 (s, 1H, triazole-CH), 8.59 (s, 1H, pyrimidine-H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz; δ 16.69, 50.79, 58.06, 66.38, 120.18, 123.32, 125.83, 126.89, 128.79, 131.81, 132.48, 133.60, 134.90, 139.16, 142.73, 154.08, 169.20; LC–MS (positive ion mode): m/z 471 (M+H)^+ for C\(_{21}\)H\(_{19}\)ClN\(_6\)O\(_3\)S.

(1-(3-Nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8d

Yield (81%); IR (KBr) (ν_{max}/cm^{-1}): 2853, 1692, 1535; \(^1\)H NMR (CDCl\(_3\), 400 MHz) δ 2.88 (s, 3H, thiophene−CH\(_3\)), 3.56 (t, 4H, morpholine-CH\(_2\)), 3.84 (t, 4H, morpholine-CH\(_2\)), 5.57 (s, 2H, CH\(_2\)), 7.78 (t, 1H, Ar-H), 8.19 (d, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 8.33 (d, 1H, Ar-H), 8.59 (s, 1H, triazole-CH), 8.62 (s, 1H, pyrimidine-H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz; δ 14.60, 48.45, 55.98, 63.81, 105.49, 105.85, 113.65, 114.00, 117.53, 120.04, 121.35, 129.74, 129.86, 138.17, 140.92, 152.30, 159.72, 160.86, 166.34; LC–MS (positive ion mode): m/z 482 (M+H)^+ for C\(_{22}\)H\(_{19}\)N\(_7\)O\(_5\)S.

(1-(3-(Trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8e

Yield (74%); IR (KBr) (ν_{max}/cm^{-1}): 2933, 2848, 1719, 1538; \(^1\)H NMR (CDCl\(_3\), 400 MHz) δ 2.83 (s, 3H, thiophene−CH\(_3\)), 3.55 (t, 4H, morpholine-CH\(_2\)), 3.85 (t, 4H, morpholine-CH\(_2\)), 5.56 (s, 2H, CH\(_2\)), 7.73 (dd, 2H, Ar-H), 7.97 (d, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.21 (s, 1H, triazole-CH), 8.60 (s, 1H, pyrimidine-H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz; δ 16.62, 50.70, 58.05, 66.28, 76.99, 117.37, 120.10, 122.18, 123.19, 123.52, 125.48, 130.53, 132.45, 137.03, 139.73, 143.58, 154.00, 162.42, 163.34, 169.14; LC–MS (positive ion mode): m/z 505 (M+H)^+ for C\(_{21}\)H\(_{19}\)F\(_3\)N\(_6\)O\(_3\)S.
(1-(3-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl5-methyl-4-(piperidin-1-yl)thieno[2,3-d] pyrimidine-6-carboxylate 8f

Yield (69%); IR (KBr) \( \nu_{\text{max}}/\text{cm}^{-1} \): 2912, 1705, 1538; \(^1\text{H}\) NMR (CDCl\(_3\), 400 MHz) \( \delta \) 2.68 (s, 3H, thiophene–CH\(_3\)), 3.48 (t, 4H, morpholine-CH\(_2\)), 3.92 (t, 4H, morpholine-CH\(_2\)), 5.55 (s, 2H, CH\(_2\)), 7.72 (t, 1H, Ar-H), 7.99 (d, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 8.21 (d, 1H, Ar-H), 8.47 (s, 1H, triazole-CH), 8.89 (s, 1H, pyrimidine-H); \(^{13}\text{C}\) NMR (CDCl\(_3\), 100 MHz) \( \delta \) 14.60, 48.45, 55.98, 63.81, 105.49, 105.85, 113.37, 113.65, 114.00, 117.55, 120.04, 121.35, 129.74, 129.86, 138.17, 140.92, 152.03, 159.72, 166.34; LC–MS (positive ion mode): m/z 455 (M+H)\(^+\) for C\(_{21}\)H\(_{19}\)FN\(_6\)O\(_3\)S.

Biological assay:

**Determination of Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of visible growth after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to antimicrobial agents and also to monitor the activity of new antimicrobial agents. MIC measurements were performed using a modified agar well diffusion method.

**Antibacterial Assay**

Antimicrobial activity of all the newly synthesized compounds were assayed against two Gram positive bacteria such as *L.bacillus* and *Staphylococcus aureus* and two Gram negative bacteria such as *P.florescensa* and *E.coli* by agar well diffusion method, 200 \( \mu \)g of the tested compounds were dissolved in 1 mL of DMSO solvent. Centrifuged pellets of bacteria from 24 h old culture containing approximately 104-106 colony forming unit (CFU) per mL was spread on the surface of Muller Hinton Agar (MHA) plates. Nutrient agar medium were prepared by suspended nutrient agar 28 g in 1 liter of distilled water, autoclaved and cooled to 45 °C, and then it was seeded with 15 mL of prepared inocula to have 106 CFU/mL. Petri dishes were prepared by pouring 10 mL of seeded nutrient agar. Wells were created in medium with the help of a sterile metallic borer and test solution was added. Experimental plates were incubated for 24 h at 37 °C. Amoxycyclav was used as standard drug for antibacterial assay.
Antifungal activity of all the newly synthesized were tested with *Aspergillus niger* and *Penicillium sp* by the poison plate technique. Tested compounds were dissolved in DMSO before mixing with potato dextrose agar (PDA). The final concentration of the compounds in the medium was fixed at 200 μg/mL. Two kinds of fungi were incubated in PDA at 25 ±1 °C for 5 days to get new mycelium for antifungal assay, and then a mycelia disk of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25 ±1 °C for 5 days. DMSO solvent was added as negative control to determine possible inhibitory activity of the solvent, while Fluconazole was used as positive control.

### Table 1. Minimum Inhibitory Concentration (MIC) in μg/ml of compounds (7a-f) and (8a-f)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram–positive bacteria</td>
<td>Gram–negative bacteria</td>
</tr>
<tr>
<td></td>
<td><em>L. bacillus</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>7a</td>
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<td>100</td>
</tr>
<tr>
<td>7b</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>7c</td>
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</tr>
<tr>
<td>7d</td>
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<td>25</td>
</tr>
<tr>
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<td>6.25</td>
</tr>
<tr>
<td>7f</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>8a</td>
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<td>25</td>
</tr>
<tr>
<td>8b</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>8c</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>8d</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>8e</td>
<td>6.25</td>
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</tr>
<tr>
<td>8f</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>Amoxyclov</td>
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<td>12.5</td>
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<tr>
<td>Fluconazole</td>
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<td>—</td>
</tr>
<tr>
<td>Control</td>
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</tr>
</tbody>
</table>
Result and discussion

Antimicrobial activity

In order to search for the potent candidate, the newly synthesized compounds 7a-f and 8a-f were evaluated for their *in-vitro* antibacterial activity against Gram–positive bacteria such as *Lacto bacillus* and *Staphylococcus aureus*, Gram–negative bacteria such as *Pseudomonas florescensa* and *Escherichia coli* and antifungal activity was carried out by fungal strains such as *Aspergillus niger* and *Penicillium sp* using agar well diffusion method. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 200 µg mL\(^{-1}\). The antibacterial screening exhibited that some of the tested compounds showed excellent inhibition against various tested microbial strains. Compounds 7a-f and 8a-f with various substituents in the phenyl ring are studied to understand the influence of electron withdrawing groups on the antimicrobial activity.

Antibacterial activity

For evaluating antibacterial activity Amoxycilav was used as the standard drug. The observed minimum inhibitory concentrations (MIC) data is presented in Table 1. As can be seen from our results, all the synthesized compounds found to be effective antibacterial activity *in-vitro* against the tested organisms. Compounds with the MIC in the range of 3.12–6.25 µg mL\(^{-1}\) are reported as potent and MIC in the range 12.5–25 µg mL\(^{-1}\) are reported with good inhibition activity. Compounds 7e, 7f and 8e exhibited impressive potent antibacterial activity against *L.bacillus* as that of the standard Amoxycilav. Compounds 7c, 8c and 8f are found to be good among the series and even comparable activity with standard as antibacterial agents against all the tested bacterial cultures. In case of 7e, 7f and 8e bearing a fluoro and tri-fluoro substituent on phenyl ring possessed strong activity. The remaining compounds of these series were found to have moderate antibacterial activity. The results showed a good structural–activity relationship. On the other hand, compounds 7a, 7b, 57d, 8a, 8b and 8d showed low activity compare with that of the standard. These results clearly indicate that the electron withdrawing substituents such as fluoro and trifluoro on phenyl ring increasing the antibacterial activity. And also compound 8c more potent antifungal activity with MIC 6.25 µg mL\(^{-1}\) against *P. species strains* and compounds 7e, 8d good antifungal activity with MIC 12.5 µg mL\(^{-1}\) against both *A. niger* and *P. species* comparing that of standard Fluconazole.
Conclusion:

In this study, we have designed and synthesized a series of novel compounds based on 1,2,3-triazolo thieno[2,3-\textit{d}]pyrimidine morpholine/piperdine derivatives successively, by using simple protocols with good yields and evaluated for their antimicrobial activity. Out of the synthesized compounds six analogues have shown MIC in the range of 6.25-12.5 µg mL\textsuperscript{-1}. The compounds 7c, 7e, 7f, 8d, 8e and 8f were found to be potent candidate than the standard. Thus the presence of electron releasing methyl and methoxy substituents on phenyl ring showed less antibacterial activity. In case of antifungal activity only few of them were showed moderate to good activity. Therefore it is concluded that the antifungal activity is of our synthesized compounds with the presence of electron withdrawing fluoro group attached to the phenyl ring has responsible for anti fungal activity.
Fig. 5.1. IR spectrum of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate 2
Fig. 5.1.1. $^1$H NMR spectrum of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate 2
Fig. 5.1.2. $^{13}$C NMR spectrum of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate 2
Fig. 5.2. IR spectrum of ethyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 4a
Fig. 5.2.1. $^1$H NMR spectrum of ethyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 4a
Fig. 5.2.2. $^{13}$C NMR spectrum of ethyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 4a
Fig. 5.3. IR spectrum of 5-methyl-4-morpholinothieno[2,3-d]pyrimidine-6-carboxylic acid 5b
Fig. 5.3.1. $^1$H NMR spectrum of 5-methyl-4-morpholinothieno[2,3-d]pyrimidine-6-carboxylic acid 5b
Fig. 5.3.2. $^{13}$C NMR spectrum of 5-methyl-4-morpholinothieno[2,3-d]pyrimidine-6-carboxylic acid 5b
Fig.5.4. IR spectrum of prop-2-yn-1-yl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 6a
Fig. 5.4.1. $^1$H NMR spectrum of prop-2-yn-1-yl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 6a
Fig. 5.4.2. $^{13}$C NMR spectrum of prop-2-yn-1-yl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 6a
Fig. 5.5. $^1$H NMR spectrum of prop-2-yn-1-yl 5-methyl-4-morpholinothieno[2,3-d]pyrimidine-6-carboxylate 6b
Fig. 5.5.1. $^{13}$C NMR spectrum of prop-2-yn-1-yl 5-methyl-4-morpholinothieno[2,3-d]pyrimidine-6-carboxylate 6b
Fig. 5.6. IR spectrum of (1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 7d
Fig. 5.6.1. $^1$H NMR spectrum of (1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl) thieno[2,3-d]pyrimidine-6-carboxylate 7d
Fig. 5.6.2. $^{13}$C NMR spectrum of (1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl) thieno[2,3-d]pyrimidine-6-carboxylate 7d
Fig. 5.6.3. LCMS spectrum of (1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 7d
Fig. 5.7. IR spectrum of (1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8c
Fig. 5.7.1. $^1$H NMR spectrum of (1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8c
Fig. 5.7.2. $^{13}$C NMR spectrum of (1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8c
Fig. 5.7.3. LCMS spectrum of (1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8c
Fig. 5.8. IR spectrum of (1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8d
Fig. 5.8.1. $^1$H NMR spectrum of (1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8d
Fig. 5.8.2. $^{13}$C NMR spectrum of (1-(3-nitropheryl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl) thieno[2,3-d]pyrimidine-6-carboxylate 8d
Fig. 5.8.3. LCMS spectrum of (1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl) thieno[2,3-d]pyrimidine-6-carboxylate 8d
Fig. 5.9. IR spectrum of (1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8e
Fig. 5.9.1. $^1$H NMR spectrum of (1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8e
Fig. 5.9.2. $^{13}$C NMR spectrum of (1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8e
Fig. 5.9.3. LCMS spectrum of (1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl 5-methyl-4-(piperidin-1-yl)thieno[2,3-d]pyrimidine-6-carboxylate 8e
References


